

Sub

WHAT IS CLAIMED IS:

1. A method of preparing differentiated cells from neural stem cells comprising the steps of:

5 (a) isolating neural stem cells from the tissue of a donor,

10 (b) proliferating the isolated neural stem cells in a first culture medium having a first growth factor to produce precursor cells, and

15 (c) differentiating the precursor cells to produce differentiated cells by culturing said precursor cells in a second culture medium having at least a second growth factor wherein said second culture medium is substantially free of said first growth factor.

2. The method of Claim 1 wherein the first growth factor is epidermal growth factor.

3. The method of Claim 1 wherein the second culture medium contains serum.

4. The method of Claim 1 wherein the second growth factor is selected from the group consisting of acidic fibroblast growth factor, basic fibroblast growth factor, ciliary neurotrophic factor, nerve growth factor, brain-derived neurotrophic factor, neurotrophin 3, neurotrophin 4, interleukins, leukemia inhibitory factor, cyclic adenosine monophosphate, forskolin, tetanus toxin, high levels of potassium, amphiregulin, transforming growth factor alpha, transforming growth factor betas, insulin-like growth factors, dexamethasone, isobutyl 3-methylxanthine, somatostatin, growth hormone, retinoic acid and platelet-derived growth factor.

20

25

30

5. The method of Claim 1 wherein said differentiated cells are neurons and said second growth factor is a ciliary neurotrophic factor.

6. The method of Claim 1 wherein said differentiated cells are mature oligodendrocytes and said second growth factor is ciliary neurotrophic factor.

7. The method of Claim 1 wherein said differentiated cells are astrocytes, said second growth factor is a ciliary neurotrophic factor, and said second culture medium is substantially free of serum.

8. The method of Claim 1 wherein said differentiated cells are neurons and said second growth factor is a brain-derived neurotrophic factor.

15 9. The method of Claim 1 wherein said differentiated cells are neurons and said second growth factor is retinoic acid.

20 10. The method of Claim 1 wherein said differentiated cells are neurons and said second growth factor is basic fibroblast growth factor.

25 11. A method of preparing precursor cells comprising the steps of:

(a) isolating neural stem cells from the tissue of a donor,

(b) maintaining the isolated neural stem cells in a first culture medium containing basic fibroblast growth factor, and

(c) proliferating the isolated neural stem cells in a second culture medium containing epidermal growth

*Sub
B2*

factor and basic fibroblast growth factor to produce precursor cells.

12. A method of preparing differentiated cells from neural stem cells comprising the steps of:

5

(a) isolating neural stem cells from the tissue of a donor,

10 (b) proliferating the isolated neural stem cells in a first culture medium having a growth factor to produce precursor cells, and

15 (c) (contacting) the precursor cells with a substrate in a second culture medium substantially free of said first growth factor.

13. The method of claim 12 wherein the substrate is selected from the group consisting of poly-L-

ornithine, collagen, fibronectin, laminin, and matrigel.

3

Add b